N-acylated sulfonamide congeners of fosmidomycin lack any inhibitory activity against DXR.

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Abstract

The antibiotic fosmidomycin (**3**) is an inhibitor of the non-mevalonate pathway for isoprenoid biosynthesis. Four analogues in which an acylated sulfonamide group is substituting for its phosphonate moiety have been synthesized in a fruitless effort to preserve one negative charge in order to increase the accompanying affinity for 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), the fosmidomycin target enzyme.

In view of the ease with which bacterial resistance against various inhibitors spreads, the development of new antibiotics remains of prime importance. Treatment of malaria likewise suffers from resistance to all available therapeutics, and the search for new inhibitors and new targets therefore should be the concern of many medicinal chemists. The non-mevalonate pathway for isoprenoid biosynthesis, not being found in higher eukaryotes,[1] therefore represents an interesting target for drug development. The most studied enzyme in this pathway is the 1-deoxy-D-xylulose 5-phosphate (DOXP, **1**, Figure 1) reductoisomerase (DXR, also abbreviated IspC), catalyzing the conversion of the xylulose derivative **1** into 2-C-methyl-D-erythritol 3-phosphate (MEP, **2**)[2-4] and is a clinically validated target. Herein the natural antibiotic fosmidomycin (**3a**) and its acetyl congener (**3b**) have been known for a long time[5] and forms the hallmark for inhibition of DXR, but its use for treatment of malaria is hampered by its unfavorable pharmacokinetic properties.[6,7] In view of highly needed possible treatments for malaria or for tuberculosis, a multitude of analogues have been synthesized over the past 20 years as recently very nicely reviewed by Masini and Hirsch.[8] Both, the sulfone and the sulfonamide carrying various alkyl or arylalkyl residues of different length in the past have been evaluated as possible isosters for the phosphonate moiety.[9] While it was envisioned that

the absence of a negative charge could have facilitated the uptake, unfortunately no significant inhibitory activity was noted. This indicates that a negative charge at the phosphonate moiety position is mandatory for enzymatic inhibition.

While the sulfone and alkylsulfonamide analogues (deliberately) lacked a negative charge, it is well-known that the NH moiety of acylated sulfonamides with their enhanced imide-like structure carries a pKa well beneath the physiological pH. Indeed, two recent reports herein refer to a pKa of around 2.0 or 2.5 for an aliphatic acyl sulfonamide.[10,11] Aminoacylated sulfonamide nucleoside analogues therefore are common knowledge isosters of the aminoacylated adenylate intermediates formed by the respective tRNA synthetases, in which the deprotonated amide is mimicking the remaining negative charge of the natural adenylate intermediate.[12] We therefore decided to corroborate this hypothesis in synthesizing the fosmidomycin analogues **4a-d** (Figure 1) and evaluating their inhibitory properties on *E. coli* DXR. We hoped this way to at least partially restore the inhibitory activity in analogy with which was noted for phosphonate monoesters[9] and the expected inhibitory activity could then be optimized in further modifying the acyl moiety.

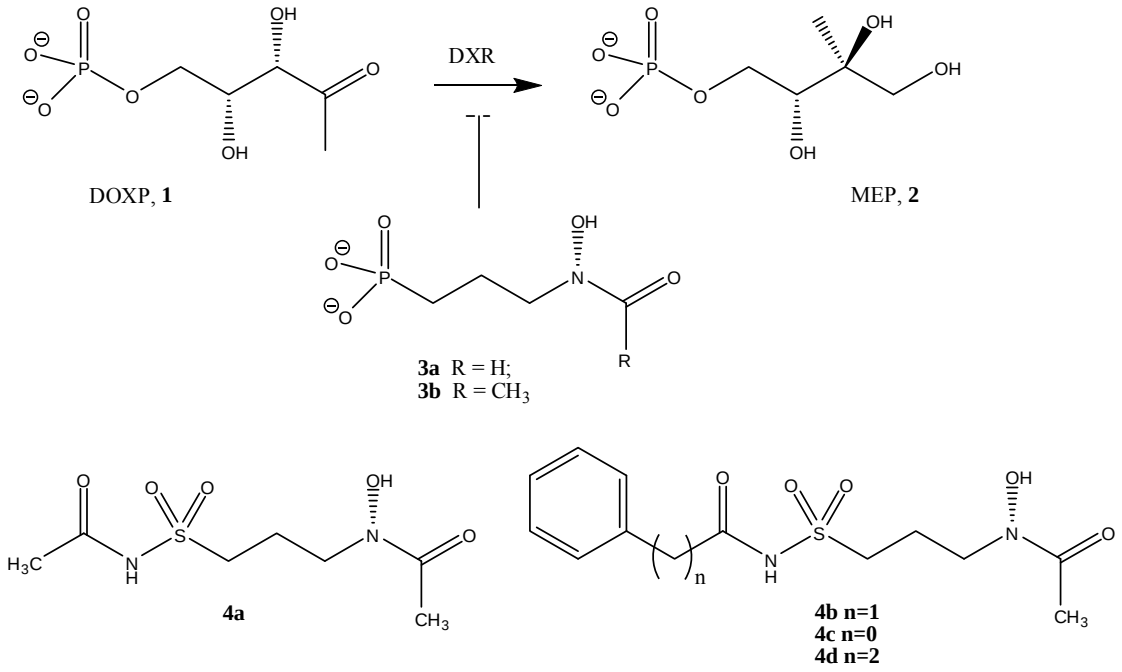
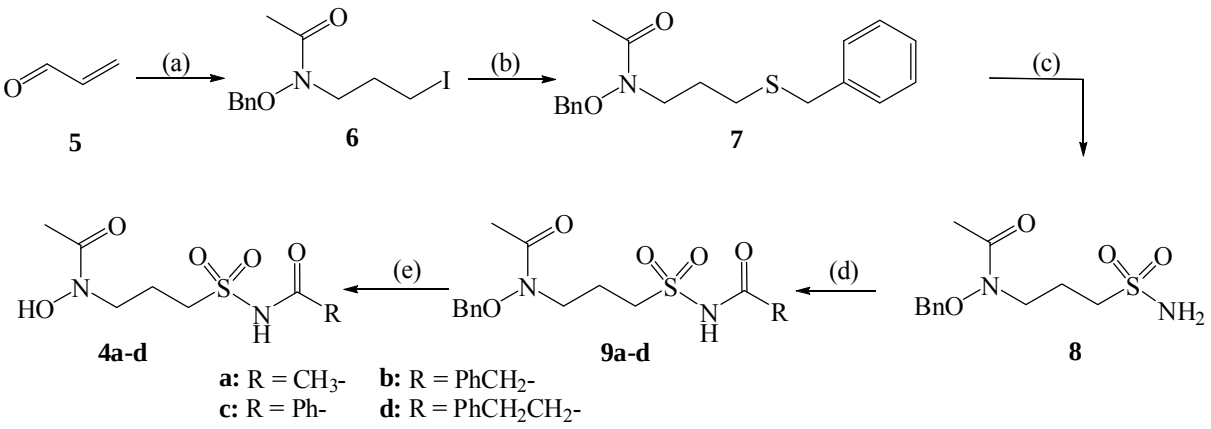


Figure 1. Inhibition of *E. coli* DXR by fosmidomycin (top) and the envisaged acylated sulfonamide mimics of fosmidomycin (bottom).



Scheme 1: reagent and conditions: (a) (i) NaI, CH_3CN, 10 min, rt (ii) TMSCl, rt, 15 min, (iii) NH_2OBn, rt, 10 min (iv) $NaBH_3CN$, TFA, rt, 1h (v) Et_3N, AcCl, rt, 30 min (one pot, 60%); **(b)** BnSNa, THF, -78ºC, 1h (90%); **(c)** ACN:AcOH:H_2O, DCDMH, 0ºC to 5ºC, 1 h; add to aq. ammonia solution (25%w/v), 0ºC to 20ºC, 1 h (96%); **(d)** acyl chloride, TEA, DMAP, dry DMF, dry DCM, 0ºC to rt (**9a**: 90%; **9b**: 39%; **9c**: 51%; **9d**: 61%); **(e)** Pd/C, methanol, H_2 atmosphere, 1-4 h (**4a**: 46%; **4b**: 46%; 4c: 50%; 4d: 42%).

Synthesis of the key intermediate **6** has been documented before and starts from acroleine (**5**) affording 60% of the iodinated precursor in a convenient one-pot reaction.[13] Conversion to the non-alkylated sulfonamide did not prove straightforward, and the iodide was smoothly converted to the benzyl thioether **7**, which upon treatment with 2,4-dichloro-5,5-dimethylhydantoin (DCDMH) was oxidatively chlorinated and in situ converted to the sulfonamide derivative **8** with aqueous ammonia in 96% yield.[14] This little known methodology using DCDMH indeed proved to be a mild and efficient procedure for the direct conversion of sulfur compounds to the corresponding arenesulfonyl chlorides in excellent yields. Acylation afforded the compounds **9a-d** which upon debenzylation gave the target compounds **4a-d** in very moderate yields.[15]

The importance of a negative charge (the phosphonate part) has been evaluated in 2008 by Perruchon *et al.*[9] and sulfonamide analogues lacking this charge proved inactive. Acylation of the sulfonamide renders the nitrogen more acidic and should result in a negative charge at physiological pH. These efforts therefore constitute a first attempt in trying to improve the affinity for the enzyme. If this hypothesis is borne out, we could start developing a new series of compounds. The new fosmidomycin mimics therefore were evaluated for their inhibitory effects on the *E. coli* DXR enzyme using a spectrophotometric assay monitoring the substrate

dependent oxidation of NAPDH. Unfortunately, even in presence of 100 μM concentrations of **4a-d**, the residual enzymatic activity remained at or close to 100%, highlighting the absence of inhibitory effects of the acylated sulfonamides for the target enzyme (supplementary file).

Besides generating a negative charge, the choice for synthesizing **4b** was inspired by the activity as reported for the phenylethyl phosphonate ester[9], where apparently the phenyl moiety can be accommodated in the active site and increases the inhibitor activity. According to our molecular modeling the acyl moiety of **4b** indeed can be placed in a larger hydrophobic pocket as was expected for the larger phosphonate esters[9] and as shown for alpha-phenyl substituted analogues of fosmidomycin.[10] In view of the absence of inhibitory activity for **4a-b** on DXR, the congeners **4c** and **4d** were synthesized, as both side chains likewise *in silico* can be accommodated in the active site as shown in the supplementary file. Based on docking (steric effects), there is thus no obvious reason why the compounds should not be active. Hence, except for a much more delocalized charge, a rationale for the lack of affinity for the compounds **4b-d** for the target enzyme is missing, but discourages therefore further exploration of this scaffold. Adding to all previous efforts, it is clear now there is little room for substituting the fosmidomycin phosphonate moiety. Recent efforts to alter the chelating moiety of the lead compound likewise proved deleterious to the inhibitory activity.[10]

The newly synthesized compounds **4a** and **4b** in addition were subjected to a broad screening panel including *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania infantum*, *Plasmodium falciparum* K1, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Trichophyton rubrum*, *Candida albicans*, *Aspergillus fumigatus* and MRC-5 cells. However, no antimicrobial activity nor any cellular toxicity could be noted at the highest test concentration of 64 μM.[16]

In conclusion, it has been corroborated that acylation of sulfonamide mimics of the fosmidomycin phoshonate part is not able to restore the inhibitory effect on *E. coli* DXR, nor are the new compounds endowed with any antibacterial or antiparasitic activity.

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Government (grant 20100225-7). A supplementary file describing all synthetic procedures and biological evaluations, with NMR copies of all intermediates is provided.

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15. Analytical data for the target compounds; Compound **4a**: ^{1}H NMR (300 MHz, CD_3OD) d 2.06 (s) and 2.10 (s, 2xCOC*H*$_3$) ,2.05-2.15 (m, C*H*$_2$, 8H in total), 3.41 (t, 2H, *J* = 7.8 Hz, C*H*$_2$SO$_2$NH), 3.73 (t, 2H, *J* = 6.6 Hz, CH_2N); ^{13}C NMR (75 MHz, CD_3OD) d 20.2 (CO*C*H$_3$), 22.2 (*C*H$_2$), 23.4 (CO*C*H$_3$), 47.4 (*C*H$_2$N), 51.2 (*C*H$_2$SO$_2$NH), 172.0 (*C*ONH), 174.1 (*C*ONH); HRMS for $C_7H_{13}N_2O_5S$ ([M-H]$^-$) calcd: 237.0551, found 237.0555; Compound **4b:** ^{1}H NMR (600 MHz, CD_3OD) d 2.07 (s, COC*H*$_3$) and 2.01-2.09 (m, C*H*$_2$, 5H in total), 3.39 (t, 2H, *J* = 7.8 Hz, C*H*$_2$SO$_2$NH), 3.62 (s, 2H, C*H*$_2$Ph), 3.69 (t, 2H, *J* = 6.6 Hz, C*H*$_2$N), 7.26-7.33 (m, 5H, Ph); ^{13}C NMR (150 MHz, CD_3OD) d 20.2 (CO*C*H$_3$), 22.2 (*C*H$_2$), 43.7 (*C*H$_2$Ph), 47.4 (*C*H$_2$N), 51.2 (*C*H$_2$SO$_2$NH), 172.8 (*C*ONH), 174.1 (*C*ONH); HRMS for $C_{13}H_{17}N_2O_5S$ ([M-H]$^-$) calcd: 313.0864, found 313.0861; Compound **4c:** ^{1}H NMR (600 MHz, CD_3OD) δ 7.92 (d, *J* = 7.3 Hz, 2H, Ph), 7.59 (t, *J* = 7.3 Hz, 1H, Ph), 7.48 (t, *J* = 7.6 Hz, 2H, Ph), 3.75 (t, *J* = 6.4 Hz, 2H, CH_2N), 3.55 – 3.49 (t, 2H, *J* = 7.2 Hz, C*H*$_2$SO$_2$NH), 2.18 – 2.08 (m, C*H*$_2$) and 2.09 (s, COC*H*$_3$) (5H in total); ^{13}C NMR (150 MHz, MeOD) δ 174.1 ($COCH_3$), 170.1 (CONH), 134.4 (Cq), 134.0, 129.6, 129.5 (5CH, Ph), 51.4 (*C*H$_2$SO$_2$NH), 49.4 (CH_2N), 47.5 ($COCH_2$), 22.3 (CH_2), 20.2 ($COCH_3$); HRMS for $C_{12}H_{15}N_2O_5S$ ([M-H]$^-$) calcd: 299.0707, found 299.0712; Compound **4d:** ^{1}H NMR (300 MHz, CD_3OD) δ 7.38–7.12 (m, 5H, Ph), 3.68 (t, *J* = 6.3 Hz, 1H, CH_2N), 3.45–3.31 (m, 2H, C*H*$_2$SO$_2$NH), 2.93 (t, *J* = 6.0 Hz, 2H, $COCH_2$), 2.62 (t, *J* = 6.6 Hz, 2H, CH_2Ph), 2.11 (s, 3H, COC*H*$_3$), 2.05-1.86 (m, 2H, C*H*$_2$); ^{13}C NMR (75 MHz, MeOD) δ 173.2 (*C*OCH_3), 172.4 (*C*ONH), 140.0 (Cq), 127.8, 127.7, 125.6 (5CH, Ph), 49.5 (*C*H$_2$SO$_2$NH), 45.6 (*C*H$_2$N), 37.4 ($COCH_2$), 30.0 (CH_2Ph), 22.9 ($COCH_3$), 20.5 (CH_2); HRMS for $C_{14}H_{19}N_2O_5S$([M-H]$^-$) calcd: 327.1020, found 327.1017.
16. Biological screening of the compounds was performed as reported in Cos, P.; Vlietinck, A. J.; Berghe, D. V.; Maes, L. *J. Ethnopharmacol* **2006,** *106*, 290.